REMARKS

Interview

Applicant very much appreciates the opportunity for a telephone interview with the Examiner on September 14, 2006. Examiner's comments have been very helpful towards advancing the prosecution of this Application. Applicant's comments on the Interview Summary are provided in a separate document accompanying this response.

Claim Amendments

Claims 1-2, 7-8; 17, 19-21, 23-26, 28-34, 38-39, and 38-39, 42-56 are currently pending in the application. Claim 11, 12, 14, 15, 18 and 36 were previously cancelled. Claims 3-6, 10, 16, 25, 27, 30 and 37 have been newly cancelled without prejudice to pursuing subject matter of these claims in this or another application. Claims 9 has been cancelled, and the subject matter added to new claims 46. Claim 13 has been cancelled, and the subject matter added to new claim 43. Claim 22 has been cancelled and the limitation of claim 22 incorporated into one or more independent claims. Claim 30 has been cancelled and the subject matter incorporated into claim 28. Claims 1, 2, 7-8, 17, 19, 20-21, 23-26, 28-29, 31-34 are newly amended; while claims 38-56 are newly added. The claim amendments find support in the specification and are discussed below.

Independent claims 1 and 2 have been amended to more closely reflect language found acceptable in co-pending application 10/268,730. Applicant has clarified that the method of diagnosis is performed in a "test subject". Support for this amendment is found throughout the specification including paragraph 13 of US 2004/0014059 (the "parent application") (hereinafter all references are to the parent application unless otherwise noted). Applicant has also amended the claims to clarify that the presence of RNA detected and quantified is in RNA "of a blood sample which has not been fractionated into cell types". Support for this phrase is found throughout the specification including paragraph 78 and is more specifically discussed in the remainder of this response. Independent claims 1 and 2 have also been amended to require

detection and quantification of RNA encoded by "two or more genes". Support for this amendment is found in paragraph 11, 34, 58 and 59 as well as Figure 4 and Figure 5. Support for other amendments within independent claims 1 and 2 have previously been provided in the file wrapper accompanying application 10/268,730.

Independent claims 7 and 8 have been amended without prejudice to the Applicant's rights to pursue the subject matter of the unamended claims in another application. Claim 7 has been amended to include reference to the use of "an oligonucleotide of predetermined sequence" which includes a primer or a probe of predetermined sequence. Claim 7 has been amended clarify "said oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by said gene" and claim 8 has been modified to clarify that "said primers being specific only for RNA, and/or cDNA complementary to said RNA, encoded by a said gene" The term oligonucleotide is found throughout the specification including paragraph 28. Support for the use of oligonucleotides of predetermined sequence is found throughout the specification at paragraph 14 paragraph 37; paragraph 55; paragraph 57, and paragraph 58. There is also support for the use of probes at paragraph 79; paragraph 46; and paragraph 30. Support for the oligonucleotides being specific only for RNA and/or cDNA complementary to said RNA, corresponding to a single gene is also found throughout the specification at paragraphs 51, 53, 55, 57, 58, and paragraph 70.

Independent claims 7 and 8 have also been amended to refer to "subjects" having said disease and "control subjects" replacing previous language contemplating "one or more subjects" and "one or more control subjects". Support for these amendments can be found throughout the specification and in particular Figure 5A and Figure 5B.

New independent claims 50, 51, 52, 53, 54 and 55 have been added which refer to "RNA extracted from unfractionated cells of a lysed blood sample". Support for this phrase is found at paragraph 52 and is discussed further herein.

Priority

The Examiner contends that in claim 7, the limitation "*detecting...in an unfractionated*

sample of whole blood" and in claim 8, the limitation "*from RNA extracted from an unfractionated sample of whole blood*" do not appear to have basis in the parent application (U.S. No. 10/268,730). The Examiner concludes that the priority date for these claims is the instant filing date, June 20, 2003. The Examiner points out that the specification nevertheless provides clear basis for (a) methods wherein cell types within blood are not fractionated (paragraph 78), (b) methods wherein a centrifuged pellet, which would clearly contain cellular matter, is treated for use in RT-PCR, and (c) methods wherein primers or probes are provided "directly" to whole blood.

Applicant respectfully disagrees that the limitations "*detecting...in an unfractionated sample of whole blood*" and "*from RNA extracted from an unfractionated sample of whole blood*" do not have basis in the parent application, and that the priority date for these claims is June 20, 2003. Nevertheless, in the interest of expediting prosecution of the instant application, Applicant currently elects to amend independent claims 1, 2, 7, 8, 17 and 19 to recite the phrase "*a blood sample which has not been fractionated into cell types*"; and has amended new independent claims 50-55 to recite the phrase "*unfractionated cells of a lysed blood sample*".

Support for reciting RNA of "*a blood sample which has not been fractionated into cell types*" can be found in the parent application, for example, at paragraph 78 (note that all recitations herein cite the pre-grant publication US2004/0014059 unless otherwise indicated), as pointed out by the Examiner in the Office Action mailed August 11, 2006. Support for the limitation RNA of "*unfractionated cells of a lysed blood sample*" can be found in the parent application, for example in Example 5 at paragraph 52, ("[a] blood sample was first treated with lysing buffer and then undergone centrifug[ation]" [and] the .."total blood cell RNA [of the] resulting pellet" [used to perform RT-PCR]). Applicant is further supported in these views by the Examiner's Interview Summary mailed September 25, 2006 that confirms the Examiner's position that both these amendments are supported in the parent application and overcome the outstanding rejections of record. In view of the above amendments and arguments, Applicant believes to have overcome the priority objections raised by the Examiner.

35 U.S.C. § 112, 1st Paragraph Rejections

The Examiner rejects Claim 9 under 35 U.S.C. § 112, 1st paragraph, as failing to comply with the written description requirement. Applicant has cancelled claim 9, but has reintroduced the subject matter of claim 9 in claim 46. The Examiner states that claim 9 contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Specifically, the Examiner contends that claim 9 includes a new limitation, "*the subjects having said disease are asymptomatic with respect to said disease,*" which appears to be new matter. The Examiner asserts that this is a generic claim encompassing the selection of markers in any possible disease, and contends "the specification does not generally describe or discuss the use of asymptomatic patients for marker identification for any or all diseases". Examiner thus rejects the claim for new matter on the basis that the breadth of the claim does not appear to be contemplated in the instant specification.

In a telephone interview held on September 14, 2006, Applicant set forth a number of arguments (described below) which, together, demonstrate that (a) the specification generically contemplates the use of blood based biomarkers to monitor asymptomatic patients of disease, and (b) the specification contemplates and enables the use of asymptomatic patients to identify biomarkers of asymptomatic disease.

Examiner has agreed both during the interview, and in the Interview Summary (dated September 25, 2006) that there is written description support in paragraph 79 of the specification for the limitation *"said subjects having said disease are asymptomatic with respect to said disease"* which is the sole limitation added to claim 46 (former claim 9). However, as introduced in the Interview Summary, Examiner now appears to be suggesting that although paragraph 79 provides written support for use of methods of the invention to diagnose subjects who are asymptomatic for disease, Applicant must also provide additional written support for a method of identifying these biomarkers using asymptomatic patients other than patients who are asymptomatic for diabetes. With respect, this greater burden is not consistent with the written description requirement codified in §112 1st paragraph. To meet the written description requirement, a patent specification must describe the claimed invention in sufficient detail that

one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. See, e.g., Moba, B.V. v. Diamond Automation, Inc., 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003); Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563, 19 USPQ2d at 1116. Applicant clearly demonstrates a recognition that the invention encompasses identifying and using biomarkers to monitor patients with asymptomatic disease as they progress through onset of overt symptoms (see paragraph 79 "*the application of presently disclosed method for detecting tissue-specific transcripts in blood....may also be used for monitoring a patient for the onset of overt symptoms of a disease*"). Applicant has also shown how to apply the method of identifying biomarkers for asymptomatic disease using the example of diabetes (Example 5 paragraph 55) and Examiner has agreed that the specification teaches the identification of the insulin gene as a biomarker of asymptomatic diabetes using subjects who were asymptomatic for diabetes (see Office Action dated August 11, 2006).

During the telephone interview, Applicant pointed to additional teachings of a method of identifying a second, non-insulin gene (ZFP) which is differentially expressed as between patients having diabetes, asymptomatic patients and normal individuals (Example 6, paragraph 58). Applicant also pointed to paragraph 58 and Figure 5B which teach that ZFP is differentially expressed as between normal and diabetic individuals and may be a biomarker of asymptomatic cardiac hypertrophy and/or heart failure since the diabetic individuals were asymptomatic for cardiac hypertrophy and heart failure. It would be clearly understood by a person skilled in the art, that in order to confirm identification of ZFP as a biomarker indicative of asymptomatic cardiac hypertrophy and/or heart failure, one would need to apply the methods of the invention (as is done for insulin and asymptomatic diabetes) to subjects who are asymptomatic for cardiac hypertrophy and/or heart failure as compared with normal subjects.

Given the above, there is sufficient written description support for the limitation "*said subjects having said disease are asymptomatic with respect to said disease*" and that the specification contemplates and enables the use of asymptomatic patients to identify biomarkers of asymptomatic disease. In view of these aforementioned comments, Applicant respectfully requests reconsideration and withdrawal of the rejection.

35 U.S.C. § 112 2nd Paragraph Rejections

The Examiner has rejected claims 7-10, 13, 16-17 and 19-37 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner has rejected the claims as being indefinite over the recitation of "*an unfractionated sample of whole blood*" which is recited in claim 7 and "*from RNA extracted from an unfractionated sample of whole blood*" in claim 8.

Applicant respectfully disagrees, nevertheless, in order to expedite prosecution of the instant application, Applicant currently elects, as described above in the response to the Examiner's priority remarks, to file instead claims which recite RNA of "*a blood sample which has not been fractionated into cell types*" and RNA of "*unfractionated cells of a lysed blood sample*".

Support for these amendments is discussed above in Applicant's response to the Examiner's priority remarks. Applicant respectfully submits that, in view of the specification and prosecution history, RNA of "*a blood sample which has not been fractionated into cell types*" clearly and unambiguously refers to RNA of unfractionated, RNA-expressing cells of blood. Thus this method is in clear contrast to RNA of a blood sample which has been fractionated into cell types which, for example is shown in Figure 5C. Figure 5C corresponds to analysis of RNA of isolated types of RNA-expressing blood cells, such as granulocytes ("GR"), T-lymphocytes ("CD3"), B-lymphocytes ("CD19") and monocytes ("MONO").

Applicant further respectfully submits that, in view of the specification and prosecution history, RNA of "*unfractionated cells of a lysed blood sample*" clearly and unambiguously refers to RNA of cells present in a blood sample following lytic treatment, and excludes any fractionation of the cells subsequent to lysing the blood sample. Support for this amendment is found at paragraph 52 with the recitation "*A blood sample was first treated with lysing buffer and then* [centrifuged, and the] *resulting pellets were processed with RT-PCR*".

The Examiner has also rejected claims 7 and 8 as being indefinite over the recitation "*said gene being detectable in said sample*". Applicant notes for the record that claim 7 actually recites "*said control RNA being encoded by said gene and being detectable in said sample*".

Examiner appears to be suggesting that the phrase in claim 7 and claim 8 are indefinite based on the contention that whether or not something is "detectable" is a latent property and could depend upon the methodology used for detection, as opposed to whether or not the gene itself is actually being expressed in the sample. Applicant respectfully disagrees that claims 7 or 8 are indefinite in this regard, however in order to expedite prosecution, Applicant has amended claim 7 and claim 8 to clarify that the control RNA has been detected by amending to the phrase "*said control RNA having been detected in said sample*". Applicant submits that these amendments clarify that said sample is one in which the control RNA has been detected, rather than merely describing an inherent property of the control RNA. Specification support for the recitation "*said control RNA having been detected in said sample*" can be found, throughout the parent specification, for example at the Title, Abstract, originally filed claim 1, and paragraph 49. In view of these aforementioned comments and amendments, Applicant respectfully requests reconsideration and withdrawal of the rejections.

35 U.S.C. § 102(b) Rejections – Ditkoff et al.

The Examiner has rejected claims 1-6 under 35 U.S.C. 102(b) as being anticipated by Ditkoff et al. (Surgery, December 1996, Vol. 120, pages 959-965).

Applicant respectfully disagrees with the contention that claims 1-6 are anticipated by Ditkoff *et al.* Regarding claim 1, the Examiner contends that Ditkoff et al. teaches a method for diagnosing or prognosing a disease in an individual comprising determining the level of expression of a gene (namely thyroglobulin) in a blood sample and detecting a difference of said level of expression of said gene in said blood sample relative to the level of expression of the same gene of a control, wherein a difference in expression levels is indicative or predictive of said disease and in particular cites p. 959-960, Table, p. 963.

Anticipation requires that a prior art reference disclose each and every limitation of the claim. *Atlas Powder Company et al. v. IRECO*, Incorporated et al., 190 F.3d 1342, 1347 (Fed. Cir. 1999).

Ditkoff et al. does not, as required by section 102, teach all the limitations of the instant

claims. There is no teaching or suggestion in Ditkoff of a method comprising the limitation that the control RNA be detected in blood samples from control subjects as required by newly amended claims 1 and 2. Furthermore, as pointed out by the Examiner, Ditkoff *et al.* is limited to teaching simple detection ("yes or no observation") of expression of a gene. Applicant submits that the phrase "determining a level" as used in the claims requires more than the simple detection of presence or absence, but rather, as would be understood by a person ordinarily skilled in the art, requires some measure of quantitation of gene expression products. Nevertheless, in order to expedite prosecution of this application, Applicant has elected to file amended claims which include the term "quantifying a level" and "said quantified level" in order to clarify this issue.

Examiner also argues that Ditkoff et al. teaches comparison of a level of gene expression in a blood sample as compared to the level of expression in a thyroid tissue sample, and the expression detected in the diseased sample is "the same" as in the thyroid tissue. Applicant has amended the claims to clarify that the control sample must also be a blood sample.

Finally Examiner argues that Ditkoff teaches the comparison of amplification products among each of the patients, teaching that all patients with metastatic disease had an amplification product for the test and suggesting that each of these patients may be considered a "diseased control" relative to the each of the other patients. Applicant would again note that Ditkoff does not teach comparing levels, either as between diseased individuals or as between diseased individuals and normal controls. As noted above, Applicant has now clarified this issue by amending independent claims 1 and 2, to include the term "quantifying".

Applicant also currently elects to amend independent claims 50 and 51 to ensure comparison is as between quantified levels of two or more genes between subjects having a disease, and control subjects.

35 U.S.C §§ 102(a) and 102(b) Rejections – Sharma et al.

The Examiner has rejected claims 1-10, 16, 17, 19-27, and 32-37 under 35 U.S.C. 102(a) and 102(b) as being anticipated by Sharma et al. ("Sharma") (WO 98/49342, as cited in IDS). In

particular Examiner claims that Sharma teaches the preparation of gene transcript patterns from unfractionated whole blood (where unfractionated whole blood is interpreted to mean the cell types within blood are not separated from one another) and teaches determining a difference in a quantified level of expression as between diseased and control subjects. With regards to the further requirement incorporated within independent claims 7 and 8 that the gene detected is one expressed in blood and in a non-blood tissue of a subject not having said disease, the Examiner indicates that this would be an inherent property of at least some of the genes that would be detected using the method of Sharma since "Sharma et al appear to be using substantially the same methods as used by the Applicant".

With respect, the Applicant disagrees that the methods taught by Sharma and those claimed by the Applicant are "substantially the same method" and further disagrees that it must therefore be an inherent property of at least some of the genes prophetically detectable using the method of Sharma be genes expressed in blood and a non-blood tissue of a subject not having said disease. To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' In re Robertson, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999).

Nevertheless, in order to expedite prosecution of this application, Applicant has elected to amend independent claims 7 to clarify that the Applicant's method uses "oligonucleotides of predetermined sequence" and has further amended claims 7 and 8 to clarify that "said oligonucleotide" of claim 7 and "said primers" of claim 8 are specific only for RNA encoded by a single gene" to further distinguish the Applicant's methodology from the methods of Sharma. Similar amendments have been made to independent claims 1, 2, 17, 19, 50, 51, 52, 53, 54, and 55. A more detailed discussion of the differences between Sharma and the Applicant's claimed methodology are discussed below.

Sharma teaches a method which requires the use of **non sequence based separation techniques** to identify RNA which is differentially expressed as between diseased

and healthy individuals. In fact, Sharma takes great pains to "teach away" from the use of sequence specific methods, addressing this issue multiple times and in absolute statements (ie there is no suggestion that the use of non sequence based separation techniques are merely preferred embodiments, although preferred embodiment language is used elsewhere within the specification). As noted In re Gurley, 27 F.3d 551 31 USPQ2d 1130 (Fed. Cir. 1994), "A reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant".

Sharma teaches that "*Separation of the normal and diseased sample mRNA or cDNA is performed... by **non-sequence based separation techniques***" (see page 12 of WO98/49342, emphasis added). Sharma proceeds to define "Non-sequence based separation techniques" as being "*techniques which allows discrimination between transcripts or their corresponding cDNAs **without involving the use of sequence information of particular transcripts to discriminate between the different transcripts/cDNA***." (pg. 13 of WO98/49342, emphasis added). Sharma further emphasizes that "*sequence based techniques, which are excluded, include for example capture with probes directed to different sequences by hybridization" (pg. 13 of* WO98/49342). Clearly, a person skilled in the art reading the teachings of Sharma would be led away from using probes which are specific to unique sequences of cDNA/mRNA.

Applicant thus disagrees that the method taught by Sharma and the method taught by Applicant are the same method and further disagrees that the method taught by Sharma is a method which would inherently identify transcripts of a single gene, let alone a gene expressed in blood and in a non-blood tissue of a subject not having said disease. Applicant further disagrees that Sharma teaches the use of oligonucleotides including primers that are specific for said gene as argued by the Examiner with respect to Applicant's claim 22.

In the telephone interview dated September 14, 2006, Applicant pointed to language as used in claim 22 of "gene specific primers" and proposed amending the claim to include "use of a gene specific primer and/or probe" to further distinguish Applicant's claimed methodology from the methodology taught by Sharma. Examiner argues that the broadest reasonable

interpretation of the term "primers and/or probe specific for a gene", includes primers and/or probe which are specific for sequences common to all mRNA such as a polyA tail or cap sequence. Applicant respectfully disagrees that the language of claim 22 can be reasonably read to include primers that bind to all genes or all products of all genes. This ignores the well understood meaning of the term "specific" and also ignores the manner in which the term "gene specific primers" is utilized within the specification. The term "gene specific primers" would be readily understood by a person skilled in the art to mean primers which hybridize specifically to a single gene (and thus products of a single gene) as compared with products of all genes. Nevertheless, in order to expedite prosecution, Applicant has amended independent claims 7, 8, 17 and 19, as well as newly added independent claims 52 -55 to clarify the language so as to exclude primers or oligonucleotides that hybridize to all genes. In claims 7, 52 and 54 Applicant has clarified that the primers or oligonucleotides are ones "being specific only for RNA, or cDNA complementary to said RNA, encoded by a single gene. In claim 8, 53 and 55, Applicant has clarified the same thing with respect to the primer set used to produce amplification products. As discussed in the previous sections, there is support throughout the specification for these amendments. In view of these aforementioned comments, Applicant respectfully requests reconsideration and withdrawal of the rejection.

35 U.S.C. § 103(a) Rejections - Nagai et al. in view of Kephart

The Examiner has rejected Claims 7, 8, 16, 20-32, 34, and 36 under 35 U.S.C. 103(a) as being unpatentable over Nagai et al. in view of Kephart (Promega Notes Magazine, Number 62, P. 11- 15, 1997).

The Examiner contends that Nagai et al. teach "a method comprising the steps of detecting the presence of RNA encoded by a gene expressed in blood and in non-blood tissue of a subject not having said disease in a sample of whole blood from each of one or more subjects having said disease, and quantifying a level of said RNA in said sample and determining a difference between said level and a quantified level of control RNA from an unfractionated sample of whole blood from each of one or more first control subjects, said control RNA being

encoded by said gene and being detectable in said sample from said control subjects, said difference identifying said gene as a marker of said disease". With respect, Applicant disagrees that Nagai teaches detecting the presence of RNA *in a sample of whole blood*, and also disagrees that Nagai teaches comparing a quantified level with a quantified level of control RNA *from an unfractionated sample of whole blood*, as these terms would be understood by a person skilled in the art.

There appears to be an inconsistency in the Examiner's recitation of the teachings of Nagai. As the Examiner rightly points out, Nagai does not teach detection of gene expression in an unfractionated sample of whole blood. Examiner may be suggesting that it is unclear what population of RNA is being detected and quantified as outlined in the Applicant's claims. In order to avoid any possible confusion Applicant has amended claim 7 to clarify that one is detecting the presence of RNA encoded by a single gene "in RNA of blood samples which have not been fractionated into cell types". The use of the clarifying phrase "in RNA of blood samples which have not been fractionated into cell types" indicates that it is the specific population of RNA found in the blood samples which have not been fractionated into cell types, which is being detected and quantified. Similarly in claim 8 it is clear that the amplification products produced are produced from "RNA of blood samples which have not been fractionated into cell types".

Applicant agrees that Nagai does not teach detection of gene expression in an unfractionated sample of whole blood. It remains true that Nagai does not teach detection of gene expression in "*RNA of blood samples which has not been fractionated into cell types* or "*in RNA of unfractionated cells of a lysed blood sample*" as the claims now read. Nagai teaches detection in RNA of isolated lymphocytes of a blood sample, of RNA encoded by the D3R gene (refer, for example to the cited reference at: Title; 1st sentence of Abstract; the "Preparation of blood lymphocytes" section under "Methods"; the 1st sentence of the "Discussion", and throughout the reference). Nagai further teaches quantifying the amount of the D3R RNA in RNA of isolated lymphocytes of blood samples of individuals having Parkinsons' and comparing with the quantified amount of D3R RNA in RNA of isolated lymphocytes of blood samples of control individuals.

The Examiner contends that Kephart provides methods to isolate RNA from whole blood (p. 11) in place of first isolating lymphocytes. In fact Kephart recommends isolating RNA from whole blood in place of first isolating mononuclear cells which include both lymphocytes and monocytes. The Examiner concludes that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the methods taught by Nagai et al. with the method as taught by Kephart so as to detect and quantify the RNA in RNA of whole blood rather than detect and quantify the RNA in RNA of lymphocytes. The Examiner concludes that the Applicant's claimed invention was prima facie obvious.

With respect, Applicant disagrees that there is a prima facie case of obviousness. In this case, the claimed invention is a method of identifying markers of disease which requires detecting and quantifying the amount of RNA present corresponding to a gene in blood samples which have not been fractionated into cell types. The detection and quantification of the amount of RNA isolated from lymphocytes is very different than the detection and quantification of the amount of RNA isolated from all of the RNA expressing cells in blood using blood samples which have not been fractionated into cell types. This is not a matter of detecting the same ultimate material, but merely saving time in the processing step prior to detection. There are numerous RNA-expressing cells of blood including leukocytes (white blood cells). Lymphocytes (including B cells and T cells) are just a small fraction of leukocytes, and actually constitute a minority of blood cells, namely representing as little as 12% and at most only 50% of the nucleated RNA-expressing cells of blood. Mononuclear cells (as referred to in Kephart) include both lymphocytes (as studied by Nagai) and monocytes. Mononuclear cells still only represent a fraction of the RNA-expressing cells. The majority of the nucleated, RNA-expressing cells of blood are granulocytes, made up mainly of neutrophils, but also including basophils, and eosinophils [refer, for example, to the enclosed "Complete Blood Count" Table. Blood Disorders/Diagnosis of Blood Disorders, In: *The Merck Manual of Diagnosis and Therapy.* Beers MH, *et al* (eds); homepage: **www.merck.com/mmhe/sec14/ch170/ch170c.html.**, see attached Appendix 1. Thus, the subject matter claimed by the Applicant is critically different than the subject matter of Nagai because there is a critical difference between the RNA-expressing cells found in a sample of

lymphocytes as compared with the RNA expressing cells found in a blood sample which has not been fractionated into cell types. A person skilled in the art would not expect that the detection and quantification of RNA products of a gene would be identical as between unfractionated blood cells and lymphocytes and thus would not consider the teachings of Kephart as relevant to the teachings in Nagai. At best, one would have to hope that the level of RNA products of a gene in one fraction of blood cells are identical to the level of RNA products of a gene in each of the other fractions of blood cells.

Applicant in fact demonstrates in Figure 5C that there is a difference between the quantity of RNA corresponding to the insulin gene as found in granulocytes ("GR"), T-lymphocytes ("CD3"), B-lymphocytes ("CD19") or monocytes ("MONO"). Furthermore, in the particular case of Parkinson disease the ordinarily skilled artisan would have considered it especially unpredictable that similar D3R gene expression differences observed in isolated lymphocytes between healthy and diseased subjects would be discovered in whole blood, due to the fact that blood of Parkinson disease patients has a particularly high proportion of neutrophils (refer, for example, to enclosed abstract of Kluter *et al.*, 1995. J Neurol Sci. 133:134), see attached Appendix #3, and a particularly low proportion of lymphocytes (refer, for example, to enclosed abstract of Marttila *et al.*, 1985. J Neurol Sci. 69:121-131), see attached Appendix #4, relative to healthy subjects.

To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

At the time of the invention one of ordinary skill in the art would not have been motivated to apply the teachings of Kephart to the findings of Nagai because the motivation offered by Kephart was not relevant to the problem at hand in Nagai. In addition, there was no reasonable expectation of success that the results obtained from analysis of RNA of <u>whole blood</u> according to the teachings of Kephart would maintain the results obtained from <u>isolated</u>

lymphocytes, as taught by Nagai et al. Namely, one of ordinary skill in the art would not have expected to detect lymphocyte-specific differential patterns of D3 dopamine receptor (D3R) expression, or any meaningful D3R expression pattern for that matter, by analysis of whole blood, such as according to the teachings of Kephart.

In view of the clear uncertainty that the ordinarily skilled artisan would have in discovering similar healthy-versus-Parkinson disease differential D3R gene expression patterns in whole blood as in isolated lymphocytes, Applicant respectfully submits that the ordinarily skilled artisan would not have been motivated to combine the teachings of Nagai et al. with those of Kephart so as to obtain the claimed invention, nor would the ordinarily skilled artisan have expected a reasonable chance of success in successfully practicing the claimed invention by combining the teachings of the cited references.

In the telephone Interview held with Applicant's representatives concerning the present application on Sept. 14, 2006, The Examiner referred to Wong *et al.*, 1997. Br J Cancer. 76:628-633 as being essentially analogous to Nagai *et al.* during and cited in the office action mailed to Applicant on Sept. 14, 2006 regarding U.S. Appl. No. 10/803,648. In fact, the results obtained by Wong *et al.* relate to gene expression patterns in peripheral blood mononuclear cells (PBMCs) obtained using Ficoll-Paque gradient centrifugation (refer to Wong *et al.* at p. 629, col. 1). As discussed above, PBMCs constitute a minority of the nucleated, RNA-expressing cells of blood, being composed almost exclusively of lymphocytes and monocytes, such cell types respectively representing only 12-50% and 2-9% of the nucleated, RNA-expressing cells of blood (refer, for example, to the enclosed "Complete Blood Count" Table"),. A diagram clarifying the type of blood cell separation obtained when performing Ficoll-Paque gradient centrifugation of blood as performed by Wong *et al.* is enclosed (Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. Fig. A.23), see attached Appendix #2. In view of the fact that PBMCs represent a structurally and functionally distinct minority of the nucleated, RNA-expressing cells of blood, similarly to isolated lymphocytes, Applicant respectfully submits that one of ordinary skill in the art, in accordance with the arguments set forth hereinabove with respect to the Nagai rejection, would not have been motivated to combine the teachings of Wong et al. with those of Kephart 1998 so as to

obtain the claimed invention, nor would the ordinarily skilled artisan have expected a reasonable chance of success in successfully practicing the claimed invention by combining the teachings of Wong *et al.* and Kephart 1998.

In view of these aforementioned comments, Applicant respectfully requests reconsideration and withdrawal of the rejection.

35 U.S.C. § 103(a) Rejections - Sharma et al. in view of Port et al.

The Examiner has rejected claim 13 under 35 U.S.C. 103(a) as being unpatentable over Sharma et al. in view of Port et al. (U.S. Pat. 5,733,728) ("Port"). Claim 13 has been cancelled, and the subject matter newly recited in claim 43. Thus, the following remarks are directed to newly added claim 43.

The Examiner contends that Sharma teaches all of the limitations of claim 13, except that of the disease being limited to heart failure. The Examiner further suggests that Sharma teaches the application of their method broadly to a variety of diseases. The Examiner claims that Port teaches that heart failure was a known human condition, and the differential expression of genes in blood tissue as a marker of heart failure had previously been disclosed by Port (see Col. 2-3).

Applicant respectfully disagrees that newly added claim 43 is made obvious by Sharma in view of Port. Applicants note that Port does not teach differential expression at the RNA level in whole blood of any gene that is indicative of heart failure. As discussed above, any detection of differential gene expression at the RNA level in one or more populations of blood cells does not necessarily correlate with the detection of RNA in whole blood, i.e. in blood samples which have not been fractionated into cell types. Similarly, Sharma does not teach quantifying differential expression at the RNA level in whole blood of any gene that is indicative of heart failure. In view of these facts, the combination of Sharma and Port does not arrive at the claimed method.

Furthermore, case law confirms that two references can not be combined in a 103 rejection, where one reference teaches away from the other. See, for example, Winner International Royalty Corp. v. Wang, 202 F.3d 1340, 53 USPQ2d 1580 (Fed. Cir. 2000) which

recognizes, if a first prior art reference "did in fact teach away from [a second reference], then that finding alone can defeat [an] obviousness objection. See also Tec Air, Inc. v. Denso Manufacturing Michigan Inc., 192 F.3d 1353, 52 USPQ2d 1294 (Fed. Cir. 1999) ("there is no suggestion to combine ... if a reference teaches away from its combination with another source").

Claim 43 is dependent upon claims 1, 2, 7, 8, 17 or 19. Claims 1, 7, and 17 are limited to a method encompassing the use of an oligonucleotide of predetermined sequence which detects and quantifies RNA encoded by a single biomarker gene or a candidate biomarker gene. Sharma teaches away from methods of identifying biomarkers useful for diagnosis that use sequence based techniques and particularly teaches away from "capture with probes directed to different sequences by hybridization" (pg. 12 of WO98/49342). Furthermore Sharma teaches that techniques that are excluded are ones "involving the use of sequence information of particular transcripts to discriminate between the different transcripts". Similarly, Claims 2, 8 and 19 require amplification of products to identify markers of /diagnose disease. Sharma teaches away from methods of identifying biomarkers useful for diagnosis that use sequenced based techniques such as amplification since these require the use of techniques "involving the use of sequence information of particular transcripts to discriminate between the different transcripts". Thus, Sharma explicitly teaches away from the methods encompassed in claims 1, 2, 7, 9, and 17 and 19, and also teaches away from the sequence based methods of identifying/diagnosing a disease such as heart failure, as taught by Port.

Applicant therefore respectfully submits that, a) in view of Port's lack of teaching that there is differential gene expression at the level of RNA in whole blood that is indicates of heart failure, and b) in view of Sharma's teaching away of sequence based methods such as those purported to be used in Port, Port can not be combined with Sharma so as to motivate the ordinarily skilled artisan to arrive at the Applicant's claimed methodology.

In view of these aforementioned comments, Applicant respectfully requests reconsideration and withdrawal of the rejection.

Double patenting rejections

While respectfully disagreeing with the contention that the claims can be rejected under double patenting rejections, Applicant will consider filing a terminal disclaimer should it be necessary upon the indication of allowable claims.

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Amy DeClouet

Respectfully submitted,

Date: Nov 14, 2006

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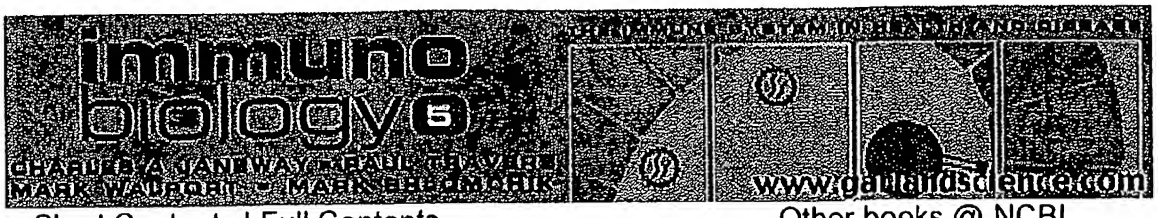
Complete Blood Count (CBC)

Test	What It Measures	Normal Values
Hemoglobin	Amount of this oxygen-carrying protein within red blood cells	Men: 14 to 17 grams per deciliter Women: 12.5 to 15 grams per deciliter
Hematocrit	Proportion of total blood volume made up of red blood cells	Men: 42 to 50% Women: 36 to 45%
Mean corpuscular volume	Average volume of individual red blood cells	86 to 98 femtoliters
White blood cell count	Number of white blood cells in a specified volume of blood	4,500 to 10,500 per microliter
Differential white blood cell count	Percentages of the different types of white blood cells	Segmented neutrophils: 34 to 75% Band neutrophils: 0 to 8% Lymphocytes: 12 to 50% Monocytes: 2 to 9% Eosinophils: 0 to 5% Basophils: 0 to 3%
Platelet count	Number of platelets in a specified volume of blood	140,000 to 450,000 per microliter
Mean platelet volume	Average volume of platelets	7 to 10 femtoliters



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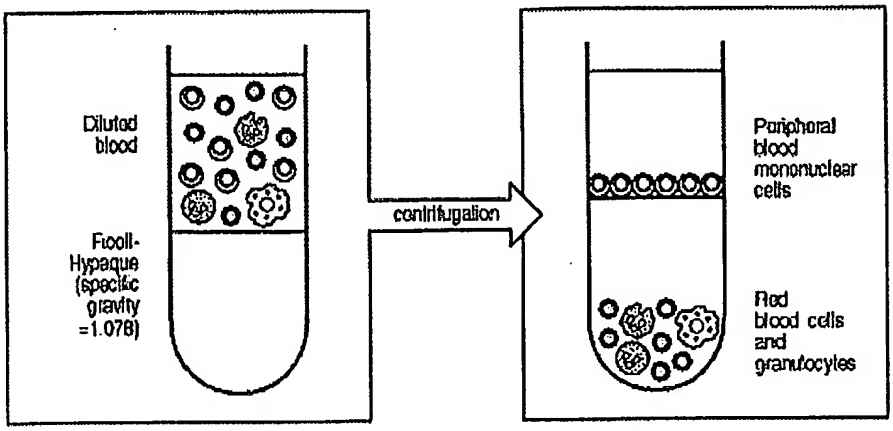

Figure A.23. Peripheral blood mononuclear cells can be isolated from whole blood by Ficoll-Hypaque™ centrifugation. Diluted anticoagulated blood (left panel) is layered over Ficoll-Hypaque™ and centrifuged. Red blood cells and polymorphonuclear leukocytes or granulocytes are more dense and centrifuge through the Ficoll-Hypaque™, while mononuclear cells consisting of lymphocytes together with some monocytes band over it and can be recovered at the interface (right panel).

J Neurol Sci. 1995 Nov;133(1-2):134-9.

Defective production of interleukin-2 in patients with idiopathic Parkinson's disease.

Kluter H, Vieregge P, Stolze H, Kirchner H.

Institute of Immunology and Transfusion Medicine, University of Lubeck School of Medicine, Germany.

The pathogenesis of Parkinson's disease (PD) is largely unknown. Recently, several studies have presented evidence of an immunological dysfunction in patients suffering from PD. We studied the immune responsiveness of patients with idiopathic PD (n = 20) by investigation of the ability of peripheral blood mononuclear cells to produce cytokines after mitogenic stimulation in a whole blood assay. A group of age-related healthy blood donors served as control (n = 19). Additionally, white blood count, leukocyte differentiation and lymphocyte subtyping were performed. PD patients had a significantly higher neutrophil count, but analysis of T-cell subsets showed no difference between the two groups. In peripheral blood, secretion of interleukin-2 (IL-2) after mitogenic stimulation was significantly diminished in the patients' group ($p < 0.01$), whereas values of IFN-alpha 2, IL-6, IFN-gamma and sIL-2R were comparable in both groups. IL-2 production correlated negatively with the mean annual dose of levodopa treatment and correlated significantly ($p < 0.002$) with amantadine uptake. Analysis of sex, age, duration of illness and other drug intake revealed no correlation with cytokine release. Our findings support the view that there is a selective abnormality in the immune repertoire of peripheral blood lymphocytes in patients suffering from PD, the reasons for which need to be explored.

PMID: 8583216 [PubMed - indexed for MEDLINE]

J Neurol Sci. 1985 Jul;69(3):121-31.

Related Articles, Links

Immune functions in Parkinson's disease lymphocyte subsets, concanavalin A-induced suppressor cell activity and in vitro immunoglobulin production.

Marttila RJ, Eskola J, Soppi E, Rinne UK.

Immune abnormalities, including the occurrence of autoantibodies against neural structures, diminished numbers of peripheral blood lymphocytes and reduced mitogen responses have been observed in patients with Parkinson's disease. In this study, we found that among the peripheral blood mononuclear cells (PBMNC) of patients with Parkinson's disease, there is a reduced number of cells spontaneously secreting immunoglobulins (Ig). After pokeweed mitogen stimulation, PBMNCs from patients with advanced Parkinson's disease had decreased capacity to elaborate Igs in vitro. In addition, there was evidence suggesting that OKT4+ (helper/inducer) subset may be decreased in Parkinson's disease along with disordered concanavalin A-induced suppressor cell activity. The findings do not primarily suggest an autoimmune pathogenesis of Parkinson's disease. The immune changes now observed partially resemble those seen in normal ageing, though being quantitatively exaggerated in Parkinson's disease. It is suggested that immune abnormalities in Parkinson's disease may result from disordered neural-immune regulation.

PMID: 3161999 [PubMed - indexed for MEDLINE]